

Listing of Claims:

This Listing of Claims replaces all the prior listings of claims.

1. (Original) A method of detecting nucleic acids with a rare mutation, wherein said rare mutation includes any change from the wildtype sequence including polymorphisms, comprising the steps of:
 - a) amplifying a nucleic acid molecule with primers flanking the rare mutation site(s);
 - b) removing the excess dNTPs after the amplification reaction;
 - c) performing a primer extension reaction using a detection primer(s) which is designed so that the 3' end of the detection primer is immediately adjacent to a nucleic acid which differentiates the wildtype from the mutant nucleic acid molecule, and at least one dNTP or ddNTP, which corresponds to a nucleoside adjacent to the detection primer in the rare mutant nucleic acid molecule; and
 - d) detecting the presence of the primer extension product(s) after the primer extension reaction and/or the consumption of dNTP,wherein the presence of a primer extension product in the reaction or the consumption of dNTP indicates the presence of the nucleic acid with a rare mutation.
2. (Original) The method of claim 1, wherein the consumption of dNTP is detected using pyrosequencing.
3. (Original) The method of claim 1, wherein only one dNTP or ddNTP corresponding to a nucleoside differentiating the rare nucleic acid variant from the more common nucleotide variant(s) is used.
4. (Original) The method of claim 3, wherein only one dNTP is used.
5. (Original) The method of claim 1, wherein a mixture of dNTP(s)/ddNTP(s) are used, wherein none of the dNTPs or ddNTPs can also be used for the extension of the wildtype DNA.

6. (Previously presented) The method of claim 1, wherein the step of detecting the presence of the primer extension product further includes measuring the amount of the primer extension product in the reaction.

7. (Canceled)

8. (Previously presented) The method of claim 1, wherein parallel primer extension reactions are performed using two different detection primers, wherein the first detection primer is designed to amplify the sense strand so that the 3' end of the primer anneals immediately adjacent to the mutation site in the sense strand and in the second reaction the detection primer is designed to amplify the antisense strand so that the 3' end of the primer anneals immediately adjacent to the mutation site in the antisense strand.

9. (Original) A method of determining the concentration or the copy number of nucleic acid molecules with rare mutations comprising the steps of:

- a) amplifying a nucleic acid sample and a known amount of a control competitive nucleic acid standard sample in the same reaction, wherein the control nucleic acid sample has been designed to have the same sequence as the rare mutation containing amplicon with the exception of one nucleic acid difference immediately adjacent to the mutation site, with primers flanking the mutation site;
- b) removing the excess dNTPs;
- c) performing a primer extension reaction using a detection primer(s), which is designed so that the 3' end of the primer anneals immediately adjacent to the rare mutation site and in the presence of at least one deoxynucleotide (dNTP) and two dideoxynucleotides (ddNTPs), wherein the dNTP corresponds to the first nucleoside after the 3' end of the detection primer in the nucleic acid with the rare mutation, the first ddNTP corresponds to the nucleoside artificially created to the control which differs from the nucleoside present in the rare mutant allele, and the second ddNTP corresponds to the nucleoside present in the rare mutant allele immediately after the mutation site;

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- d) detecting the production of primer extension products and/or consumption of ddNTP;
and
- e) determining the ratio of the amplified rare mutant, wherein the rare mutant includes any change from the wildtype including polymorphisms and the standard competitor and calculating the concentration or copy number of the rare mutant nucleic acid variant in the original sample base on the known amount of the competitor initially added to the amplification reaction in the step a).

10. (Original) The method of claim 9, wherein a mixture of dNTP(s)/ddNTP(s) are used, wherein none of the dNTPs or ddNTPs can also be used for the extension of the wildtype DNA, and the extension product from the rare mutant and the control DNA can be distinguished.

11. (Original) The method of claim 9, wherein the consumption of ddNTPs is quantified.

12. (Canceled)